

**INTERACTION BETWEEN THE RENIN ANGIOTENSIN SYSTEM AND
 α_1 -ADRENERGIC RECEPTORS AT THE RENAL RESISTANCE VESSELS
IN DIABETES MELLITUS AND HYPERTENSION**

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IN DIABETES MELLITUS AND HYPERTENSION**

by

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LIST OF ABBREVIATIONS

α	alpha
β	beta
γ	gamma
μg	microgram
2K1C	two kidney one clip
5-Meu	5-methylurapidil
7-NI	7-nitroindazole
ANOVA	analysis of variance
Ang	angiotensin
Ang I	angiotensin I
Ang II	angiotensin II
ACE	angiotensin converting enzyme
ACEi	angiotensin converting enzyme inhibitor
ACTH	adrenocorticotrophic hormone
AT	angiotensin II receptor subtype
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ARB	angiotensin receptor blocker
BP	blood pressure
BK	bradykinin
BW	body weight
cAMP	cyclic adenosine monophosphate
CVLM	caudal ventrolateral medulla
CBF	cortical blood flow
CCB	calcium channel blocker
CEC	chloroethylclonidine
cGMP	cyclic guanine monophosphate
CHF	congestive heart failure
CVD	cardiovascular disease
DAG	diacylglycerol
DLosSHR	diabetic losartan treated SHR
DLosWKY	diabetic losartan treated WKY
DM	diabetes mellitus
DPerSHR	diabetic perindopril treated SHR
DPerWKY	diabetic perindopril treated WKY
DSHR	diabetic SHR
DVR	descending vasa recta
DWKY	diabetic WKY

ECDCDM	The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus
ERK 1/2	extracellular signal-regulated kinase 1 and 2
ESRF	end stage renal failure
<i>et al.</i>	and others
F1-SHR/WKY	first generation of cross breeding of SHR and WKY rats
F2-SHR/WKY	offspring of F1-SHR/WKY rats inbreeding
g	gram
GDM	gestational diabetes mellitus
GFR	glomerular filtration rate
HCO ₃ ⁻	hydrogen bicarbonate ions
I cells	intercalated cells
i.p.	intraperitoneally
i.v.	intravenously
IGF	insulin-like growth factor
IP ₃	inositol triphosphate
JGC	juxtaglomerular cell
kg	kilogram
L-NAME	N(G)-nitro-L-arginine methyl ester
MAP	mean arterial pressure
MBF	medullary blood flow
ME	methoxamine
mEA	muscular efferent arterioles
mg	milligram
mg.dl ⁻¹	milligram per deciliter
mg.kg ⁻¹	milligram per kilogram
ml	milliliter
ml.min ⁻¹ .kg ⁻¹	milliliter per minute per kilogram
mmHg	millimeter mercury
MMP	matrix metalloproteinase
mmol.dl ⁻¹	millimolar per deciliter
mRNA	messenger ribonucleic acid
n	number of animals
NA	noradrenaline
NdLosSHR	nondiabetic losartan SHR
NdLosWKY	nondiabetic losartan WKY
NdPerSHR	nondiabetic perindopril treated SHR
NdPerWKY	nondiabetic perindopril treated WKY
NdSHR	nondiabetic SHR
NdWKY	nondiabetic WKY
NEP	neutral-endopeptidase

ng	nanogram
NO	nitric oxide
NOS	nitric oxide synthase
NTS	nucleus of solitary tract
P-cell	principal cells
PCP	prolyl-carboxypeptidase
PDCF	platelet-derived growth factor
PDE	phosphodiesterase
PE	phenylephrine
PEP	prolyl-endorphin
PG	plasma glucose
PGE ₂	prostaglandin E ₂
PGF _{2α}	prostaglandin F _{2α}
P _{gc}	glomerular capillary pressure
PIP ₂	phosphatidylinositol biphosphate
PKC	protein kinase C
PNa ⁺	plasma sodium
RAS	renin angiotensin system
RANTES	Regulated upon Activation, Normal T-cell Expressed, and Secreted
RBF	renal blood flow
RNS	renal nerve stimulation
RPP	renal perfusion pressure
RSNA	renal sympathetic nerve activity
RT-PCR	reverse transcriptase-polymerase chain reaction
RVLM	rostral excitatory region of the ventrolateral medulla
RVR	renal vascular resistance
SD	Sprague Dawley
SHR	spontaneously hypertensive rat
SNS	sympathetic nervous system
STZ	streptozotocin
tEA	thin efferent arterioles
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TGF	tubuloglomerular feedback
TGF	tumor growth factor
UNa ⁺	urinary sodium
UO	urine output
WHO	World Health Organization
WI	water intake
WKY	Wistar Kyoto

**INTERAKSI ANTARA SISTEM RENIN ANGIOTENSIN DAN RESEPTOR
ADRENERGIK- α_1 PADA SALURAN DARAH BERINTANGAN GINJAL DALAM
DIABETIS MELITUS DAN HIPERTENSI**

ABSTRAK

α_1 -adrenoseptor dan reseptor subjenis angiotensin jenis 1 (AT_1) memainkan peranan penting dalam pengawalaturan hemodinamik ginjal pada peringkat vaskulatur rintangan ginjal. Ianya memang diketahui yang diabetes melitus mampu merosakkan salur darah dan saraf periferi, dan ini diburukkan lagi oleh keadaan darah tinggi. Kajian ini dilakukan bagi mengkaji peranan fungsi subjenis α_1 -adrenoseptor dan reseptor AT_1 pada peringkat vaskulatur rintangan ginjal dalam diabetes melitus, darah tinggi, dan gabungan kedua-dua keadaan patologikal, dan sebarang kemungkinan interaksi fungsi intrarenal antara α_1 -adrenoceptors dan renin angiotensin setempat. Tikus normotensif WKY dan SHR telah digunakan. Diabetes mellitus diaruh dengan satu dos tunggal streptozotosin pada 55 mg.kg^{-1} secara intraperitoneal. Berat badan, jumlah air diminum, jumlah air kencing, paras glukosa dan natrium darah, dan tahap penyingkiran natrium melalui air kencing dimantau. Semasa kajian akut, tikus-tikus ini dibius dengan natrium pentobarbiton pada 60 mg.kg^{-1} secara intraperitoneal. Lanjutan trakeostomi untuk membolehkan pernafasan berbantu, vena jugular kiri dan arteri karotid kanan dimasukkan tiub bagi tujuan pemberian berterusan air salin dan pengukuran tekanan darah arteri purata. Selesai melakukan pembelahan abdomen garis tengah, organ dalaman abdomen dialihkan secara berhati-hati ke sebelah kanan bagi mendedahkan ginjal kiri. Satu prob pengaliran elektromagnetik dipasang pada arteri renal untuk pengukuran pengaliran darah ginjal. Elektrod dwipolar digunakan untuk merangsang saraf renal. Sebaik sahaja pembedahan selesai, 2 ml salin diberi secara intravena sebagai primer, dan tikus distabilkan untuk tempoh satu jam. Pengurangan pengaliran darah ginjal terhadap rangsangan saraf elektrik (1, 2, 4, 6, 8 dan 10 Hz pada 15V, 2 ms), dos bolus fenilefrin (0.25, 0.50, 1.00 dan 2.00 μg), metoksamin (0.50, 1.00, 2.00

dan 4.00 μg) dan angiotensin II (2.50, 5.00, 10.00 dan 20.00 ng) ditentukan dalam kehadiran 5-metilurapidil (5.00 dan 10.00 $\mu\text{g.kg}^{-1}$ termasuk 1.25 dan 2.50 $\mu\text{g.kg}^{-1}.\text{hr}^{-1}$), kloroetilklonidin (5.00 dan 10.00 $\mu\text{g.kg}^{-1}$ termasuk 1.25 dan 2.50 $\mu\text{g.kg}^{-1}.\text{hr}^{-1}$), BMY7378 (100.00 dan 200.00 $\mu\text{g.kg}^{-1}$ termasuk 25.00 dan 50.00 $\mu\text{g.kg}^{-1}.\text{hr}^{-1}$), amlodipin (200.00 dan 400.00 $\mu\text{g.kg}^{-1}$ termasuk 50.00 dan 100.00 $\mu\text{g.kg}^{-1}.\text{hr}^{-1}$), perindopril (0.20 mg.kg^{-1}) dan losartan (10.00 mg.kg^{-1}), dan tekanan darah arteri purata dimantau. Data, purata \pm s.e.m., dianalisis dengan ANOVA-satu hala atau -dua hala diikuti dengan post-hoc Bonferroni pada tahap signifikan 5%. Pengurangan secara signifikan dalam berat badan, peningkatan ketara air diminum dan penghasilan air kencing diperhatikan di dalam haiwan diabetik berbanding dengan haiwan tidak diabetik. Nilai asas tekanan darah arteri purata kekal konsisten sepanjang kajian kecuali di dalam beberapa kumpulan, manakala nilai asas pengaliran darah renal kekal tidak berubah di dalam kesemua kumpulan ujikaji. Dari respon vasokonstriktor renal, subjenis α_{1A} -adrenoseptor dan reseptor pos-sinaptik AT_1 memainkan peranan penting dalam mengawalatur vasokonstriksi salur darah rintangan dalam tikus WKY dan SHR bukan diabetik dan diabetik. Fungsi adrenoseptor subjenis α_{1B} and α_{1D} , dan Ang II beraliran amat dipengaruhi oleh keadaan penyakit diabetis melitus dan tekanan darah tinggi. Dalam tikus nondiabetik WKY, interaksi di antara sistem renin angiotensin dan adrenoseptor subjenis α_1 berlaku secara utama melalui adrenoseptor subjenis α_{1B} dan reseptor AT_1 , dan di bawah pengaruh diabetis melitus dan tekanan darah tinggi adrenoseptor subjenis α_{1A} dan α_{1D} menjadi lebih terlibat di dalam interaksi ini. Ang II beraliran mempunyai kesan lebih ketara ke atas respon dimediasi oleh adrenoseptor α_1 berbanding oleh reseptor AT_1 . Kesimpulannya, interaksi dalam ginjal di antara reseptor adrenergik α_1 dan reseptor AT_1 wujud pada peringkat salur darah rintangan ginjal, dan ini dipengaruhi secara ketara oleh keadaan penyakit diabetis melitus dan tekanan darah tinggi.

**INTERACTION BETWEEN THE RENIN ANGIOTENSIN SYSTEM AND
 α_1 -ADRENERGIC RECEPTORS AT THE RENAL RESISTANCE VESSELS
IN DIABETES MELLITUS AND HYPERTENSION**

ABSTRACT

α_1 -adrenoceptors and angiotensin type 1 (AT₁) receptor subtype play important roles in the regulation of renal haemodynamic at the level of renal resistance vasculature. It is known that diabetes mellitus could damage the peripheral blood vessels and nerves, and is worsen by hypertensive state. This study was designed to examine the functional role of α_1 -adrenoceptor subtypes and AT₁-receptor at the renal vasculature resistance in diabetes mellitus, hypertension and combination of both pathological states, and any possible intrarenal functional interaction between the α_1 -adrenoceptors and local renin-angiotensin systems. Normotensive WKY and SHR rats were utilized. Diabetes mellitus was induced by a single dose of streptozotocin at 55 mg.kg⁻¹ intraperitoneally. Body weight, water intake, urine output, plasma glucose and sodium levels, and urinary sodium excretion were monitored. During the acute study, the rats were anaesthetized with pentobarbitone sodium at 60 mg.kg⁻¹ intraperitoneally. Following tracheostomy to allow facilitated breathing, the left jugular vein and the right carotid artery were cannulated for continuous infusion of saline and measurement of the mean arterial blood pressure, respectively. Having performed a midline abdominal incision, the internal abdominal organs were carefully displaced to the right side to expose the left kidney. An electromagnetic flow probe was placed on the renal artery for renal blood flow (RBF) measurement. Bipolar electrodes were used to stimulate renal nerve. Upon completion of surgery, 2 ml of saline was given intravenously as a primer, and one hour period of stabilization was observed. Reductions in RBF to electrical stimulation (1, 2, 4, 6, 8, and 10 Hz at 15V, 2 ms), bolus doses of phenylephrine (0.25, 0.50, 1.00 and 2.00 μ g), methoxamine (0.50, 1.00, 2.00 and 4.00 μ g) and angiotensin II (2.50, 5.00, 10.00 and 20.00 ng) were determined in the

presence of 5-methylurapidil (5.00 and $10.00 \mu\text{g.kg}^{-1}$ plus 1.25 and $2.50 \mu\text{g.kg}^{-1}.\text{hr}^{-1}$), chloroethylclonidine (5.00 and $10.00 \mu\text{g.kg}^{-1}$ plus 1.25 and $2.50 \mu\text{g.kg}^{-1}.\text{hr}^{-1}$), BMY7378 (100.00 and $200.00 \mu\text{g.kg}^{-1}$ plus 25.00 and $50.00 \mu\text{g.kg}^{-1}.\text{hr}^{-1}$), amlodipine (200.00 and $400.00 \mu\text{g.kg}^{-1}$ plus 50.00 and $100.00 \mu\text{g.kg}^{-1}.\text{hr}^{-1}$), perindopril (0.20 mg.kg^{-1}) and losartan (10.00 mg.kg^{-1}), and mean arterial pressure (MAP) were monitored. Data, means \pm s.e.m., were analyzed with one- and two-ways analysis of variance followed by Bonferroni post hoc with the significance level of 5%. Significant reductions in body weight, higher water intake and urine output were observed in diabetic as compared to nondiabetic animals. Baseline values of MAP remained consistent throughout study except in few groups, whereas the RBF values remained unaltered in all experimental groups. From the renal vasoconstrictor responses, the α_{1A} -adrenoceptor subtype and postsynaptic AT_1 -receptors are functionally important in mediating the vasoconstriction of the resistance vessels in nondiabetic and diabetic WKY and SHR rats. Functionality of α_{1B} - and α_{1D} -adrenoceptor subtypes and circulating Ang II were greatly influenced by the pathological states of diabetes mellitus and hypertension. In nondiabetic WKY, the interaction between the renin angiotensin system and α_1 -adrenoceptor subtypes occurred mainly through α_{1B} -adrenoceptor subtype and AT_1 -receptor, and under the influence of diabetic and hypertensive conditions, the α_{1A} - and α_{1D} -adrenoceptor subtypes became increasingly involved in the interaction. Circulating Ang II exerted greater effects on the responses mediated by α_1 -adrenergic receptors than by AT_1 -receptors. In conclusion, intrarenal interaction between the α_1 -adrenergic receptors and AT_1 -receptor exists at the level of renal resistance vessels, and this is greatly influenced by the pathological conditions of diabetes mellitus and hypertension.

CHAPTER 1

INTRODUCTION

The maintenance of cardiovascular homeostasis represents a balance between the volume of blood in the vascular system, the pumping effectiveness of the heart, the regulation of arterial vascular resistance and the function of the kidney to ensure appropriate level of fluid reabsorption and excretion. Dysfunction of any one element of this cardiovascular-renal control interaction can be responsible for the development of a chronically elevated blood pressure (BP), or hypertension which exists in up to 20% of the adult population of most developed nations (Genest, 1994). The sustained elevated BPs impose a burden on the cardiovascular system, in particular on arteries, arterial resistance vessels, the cerebrovascular circulation, the heart and the kidneys, and places these individuals at increased risk of target-organ damages, such as stroke, myocardial infarction, left ventricular hypertrophy, heart failure and end-stage renal failure (ESRF) (Chobanian *et al.*, 2003).

A dramatic increase in patients with ESRF has been observed. In 1991, about 190,000 persons in the United States either underwent dialysis or received a renal transplant for ESRF (Klag *et al.*, 1996), and the major underlying causes were diabetic nephropathy and high BP. High BP is a strong independent risk factor for ESRF. The increase in risk associated with higher BP is graded and continuous throughout the distribution of blood pressure readings above the optimal level. The risk of ESRF in men with hypertension, as compared with men with optimal levels of BP, increases with each of the four successively more severe stages of hypertension. Systolic BP before treatment is a stronger predictor of end-stage renal disease than is diastolic pressure. Overall, the rates of ESRF are markedly higher for men with hypertensive levels of both systolic and diastolic BP (Klag *et al.*, 1996). Observational and clinical

trials involving patients with renal insufficiency have also demonstrated that lowering BP preserves renal function.

The central role of the kidney in the regulation of BP in many pathophysiological conditions has been supported by the experimental work of Guyton (1989) and the clinical evidence of Hollenberg (1980) who reported that in young essential hypertensive patients, up to two thirds could be shown to have either, or both, a slight reduction in renal blood flow or defective ability to excrete a saline volume load. Among the contributable factors are increased activity of the renin-angiotensin system and the renal sympathetic nervous system. Over-activation of these systems leads to retention of sodium and water, and increased vasoconstriction of peripheral resistance vessels that increases the vascular resistance against the blood flow, and thus the BP.

1.1 The kidney

The kidney is one of the vital organs of the body, and plays important roles in maintaining the internal environment of the body within its optimal condition. The kidney performs a variety of functions as below:

1. Regulation of water, inorganic ion balance and internal environment,
2. Removal of metabolic waste products, such as urea, uric acid and creatinine, from the blood and their excretion in the urine,
3. Removal of foreign chemicals, such as drugs, pesticides, food additives, from the blood and their excretion in the urine,
4. Synthesizing glucose from amino acids and other precursors to be released into the blood during prolonged fasting (gluconeogenesis),
5. Production of hormones/enzymes:
 - Erythropoietin, which controls erythrocyte production (erythropoiesis),
 - Renin, an enzyme that controls the formation of angiotensin and involves in the regulation of blood pressure and sodium balance,
 - 1,25-dihydroxyvitamin D, which influences calcium balance.

1.1.1 The nephron

The single functional unit of the kidney is nephron, and there are approximately 1 million nephrons in each kidney. Each nephron is made of a renal corpuscle and a tubule that extends from the renal corpuscle. The renal corpuscle is the initial filtering component of the kidney, forming filtrate from blood that is free from cells and protein. The filtrate enters the tubule through which substances are added to or removed from it. The remaining fluid at the end of each nephron drains into the collecting ducts and exits the kidney as urine.

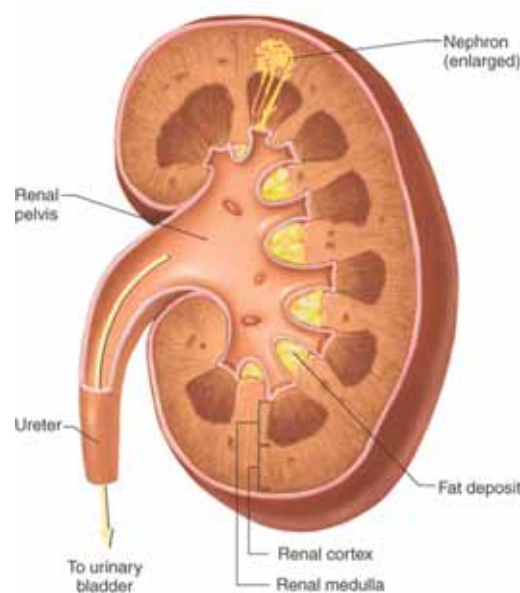


Figure 1.1: Gross anatomy of the kidney
[Adapted from Widmaier *et al.*, 2006]

1.1.1.a Renal Corpuscle

Each renal corpuscle is constituted by *glomerulus* (also called glomerular capillaries) and *Bowman's capsule* (Figure 1.2). The glomerulus is a compact tuft of interconnected capillary loops with a diameter of approximately 200 μ m, and the Bowman's capsule is a fluid-filled capsule into which the glomerulus protrudes into. The part of Bowman's capsule in contact with the glomerulus becomes pushed inwards without touching the opposite side of the capsule, forming a fluid-filled space, known as *Bowman's space*, within the capsule. As blood flows through the glomerulus from the

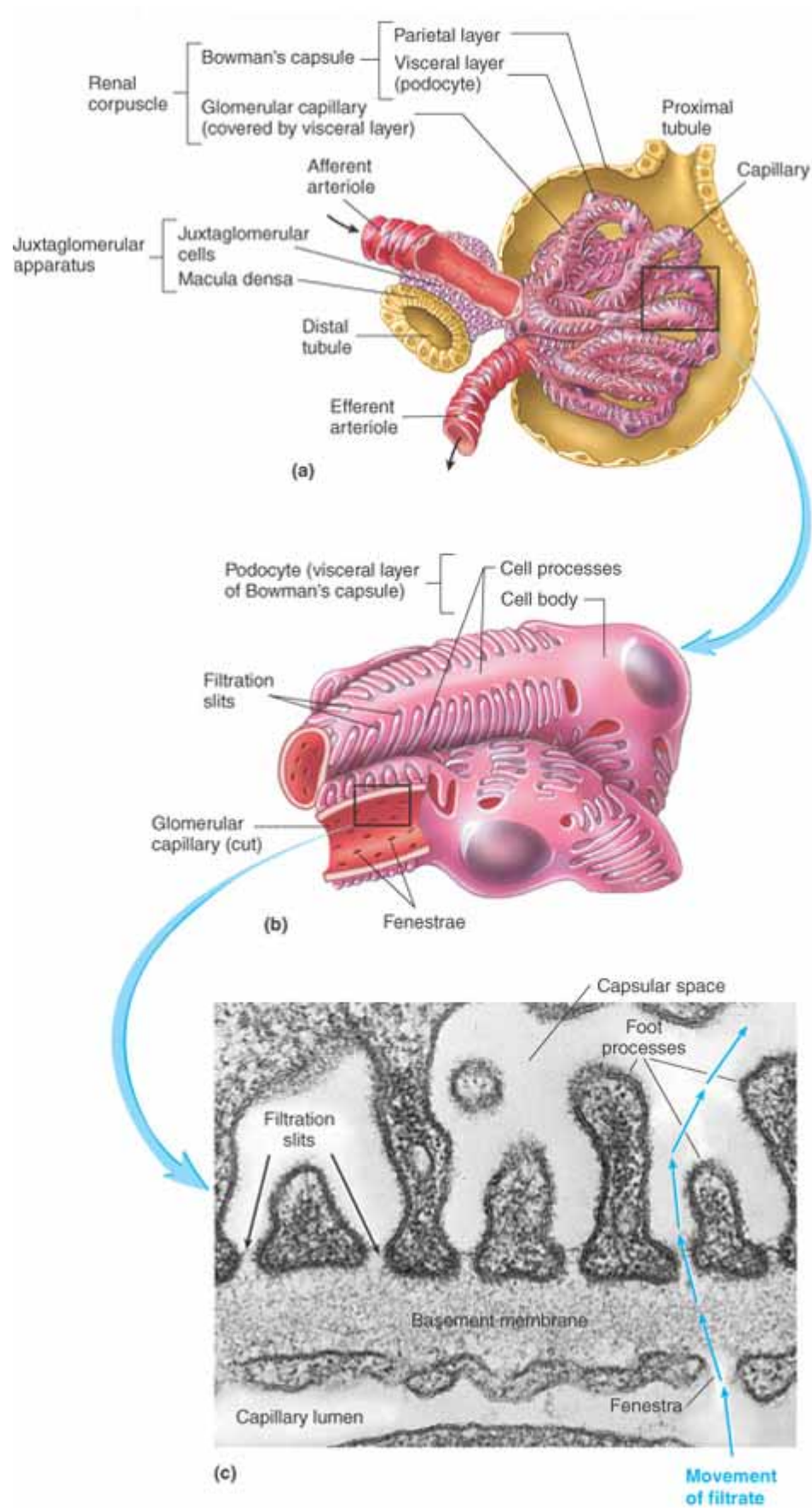


Figure 1.2: Renal corpuscle and the ultrafiltration process
[Adapted from Widmaier *et al.*, 2006]

afferent arteriole, about 20 percent of the protein-free plasma filters into Bowman's space while the remaining blood leaves the glomerulus via the efferent arteriole.

A filtration barrier separates the blood in the glomerulus from the glomerular filtrate in Bowman's space. This physical barrier is made up of three layers, namely the single-celled capillary endothelium, basal lamina (a noncellular proteinaceous layer of basement membrane, and podocytes (a single-celled epithelial lining of Bowman's capsule). The podocytes are specialised cells that have an octopus-like structure with a large number of foot processes. The fluid is filtered through the endothelial cells, the basal lamina, and finally between the foot processes of the podocytes (Widmaier *et al.*, 2006).

1.1.1.b The tubules

The tubule is continuous with a Bowman's capsule. It is hollow and cylindrical in shape with a single layer of epithelial cells. As illustrated in Figure 1.3, the tubule is divided into few segments, namely the proximal tubule, the loop of Henle, and the distal convoluted tubule. Fluid course from the distal convoluted tubule into the collecting duct system that is made up by the cortical collecting duct and medullary collecting duct. Finally, the urine drains into the kidney's central cavity, the renal pelvis, which is continuous with the ureter (Widmaier *et al.*, 2006).

The proximal tubule comprises of a convoluted portion and a straight portion. The luminal surface of the epithelial cells of the proximal tubule is lined by microvilli to enhance reabsorption processes. The loop of Henle is a sharp, hairpin-like loop comprising a descending limb and an ascending limb (Widmaier *et al.*, 2006). In the collecting ducts, principal cells (P cells) and intercalated cells (I cells) are part of the epithelial cells with the P cells predominating. The P cells involve in the Na^+ ion and vasopressin-stimulated water reabsorption, whereas the I cells are responsible for H^+ secretion and HCO_3^- ion transport (Ganong, 1999).

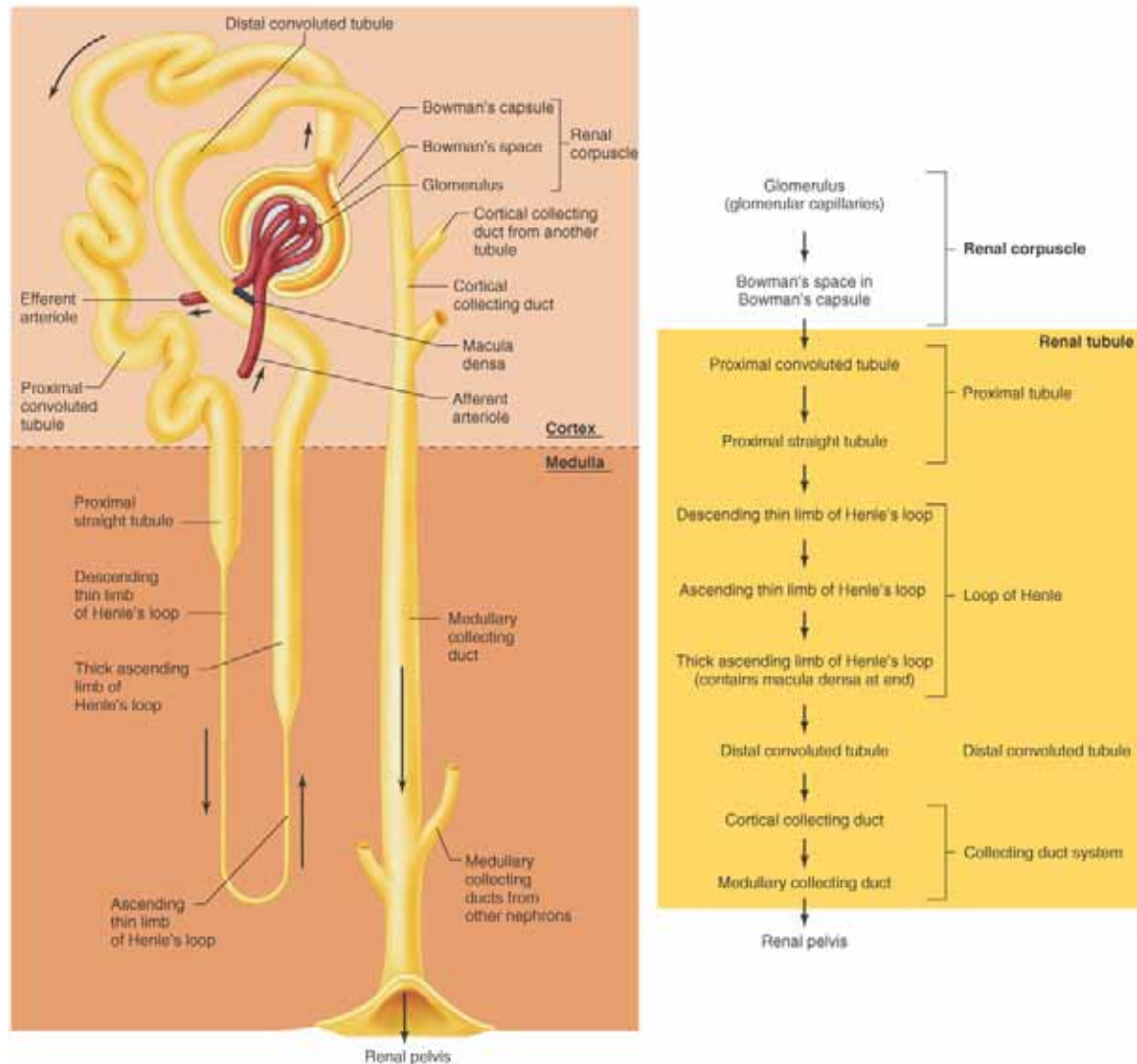


Figure 1.3: Nephron
 [Adapted from Widmaier *et al.*, 2006]

There are two important regions in the kidney. The outer region is called the renal cortex, and the inner region is the renal medulla that is further divided into three zones, namely the outer stripe of the outer medulla, the inner stripe of the outer medulla, and the inner medulla. All the renal corpuscles are located within the cortex whereas the loops of Henle extend at varying lengths from the cortex down into the medulla giving rise to two different types of nephrons, the cortical and juxtamedullary nephrons. Cortical nephrons are the majority type with 85% share. Their renal corpuscle are located in the outer cortex, and their loops of Henle are short and do not penetrate deep into the medullary region. On the other hand, the renal corpuscles of the juxtamedullary nephrons are found closest to the cortical-medullary junction, and their loops extend deep into the medulla. These long extending loops produce an osmotic gradient by generating a hypertonic medullary interstitium for the reabsorption of water (Widmaier *et al.*, 2006).

1.1.2 Renal circulation

The kidney is a highly vascularised organ, and it receives approximately a quarter of the cardiac output, which is about $1200 - 1300 \text{ ml.min}^{-1}$ of blood. All the four different regions of the kidney are perfused not equally; average total tissue blood flow in the renal cortex is $700 \text{ ml.min}^{-1}.100 \text{ g}^{-1}$, near the junction of the cortex and the outer medulla is $300 \text{ ml.min}^{-1}.100 \text{ g}^{-1}$, in the inner stripe of the outer medulla is $200 \text{ ml.min}^{-1}.100 \text{ g}^{-1}$, and in the inner medulla is between 50 to $100 \text{ ml.min}^{-1}.100 \text{ g}^{-1}$ (Aukland, 1980; Knox *et al.*, 1984; Cupples, 1986). The blood enters the kidney through the renal artery that arises from the abdominal aorta and leaves the kidney by the renal vein, both at the hilum, respectively. The renal artery branches out into the smaller interlobar artery, the arcuate artery, the interlobular artery, the afferent arteriole, the glomerulus, the efferent arteriole, and peritubular capillary. From here onwards, the blood vessels merge to form bigger vessels, the interlobular veins and eventually the renal vein (Widmaier *et al.*, 2006).

The renal circulation is unique in such a way that it includes two sets of arterioles (afferent and efferent arterioles) and two sets of capillaries (glomerulus and peritubular capillaries). The afferent arterioles (preglomerular arterioles) are short branches of interlobular arteries and they divide into capillary vessels to form the glomerulus. The capillaries coalesce to form the efferent arterioles (postglomerular arterioles), which in turn break up into either the peritubular capillaries that surround the tubules in the cortical nephron or form long hairpin loops of capillaries, known as vasa rectae, that loop deeply into the medulla along side the loops of Henle of the inner cortical and juxtamedullary nephron (Ganong, 1999; Widmaier *et al.*, 2006). As illustrated in Figure 1.4, the efferent arterioles of the juxtamedullary nephrons enter the outer stripe of the outer medulla and divide into vasa rectae that descend into the inner stripe of the outer medulla and form vascular bundles. The descending vasa rectae (DVR) in the centre of the bundles continue into the inner medulla whereas the DVR on the outer margins of the bundles give rise to a capillary plexus between the vascular

bundles in the outer medulla. The DVR found in either the outer or inner medulla divide and eventually coalesce into ascending vasa recta that carry reabsorbed solutes and water from the medulla back into the venous circulation (Kriz, 1981). Approximately 90% of the renal blood flow remains in the renal cortex and perfuses the peritubular capillary bed. The remaining 10% of the blood flow perfuses the renal medulla through the vasa rectae (Kriz, 1981; Zimmerhackl *et al.*, 1987; Cupples *et al.*, 1988).

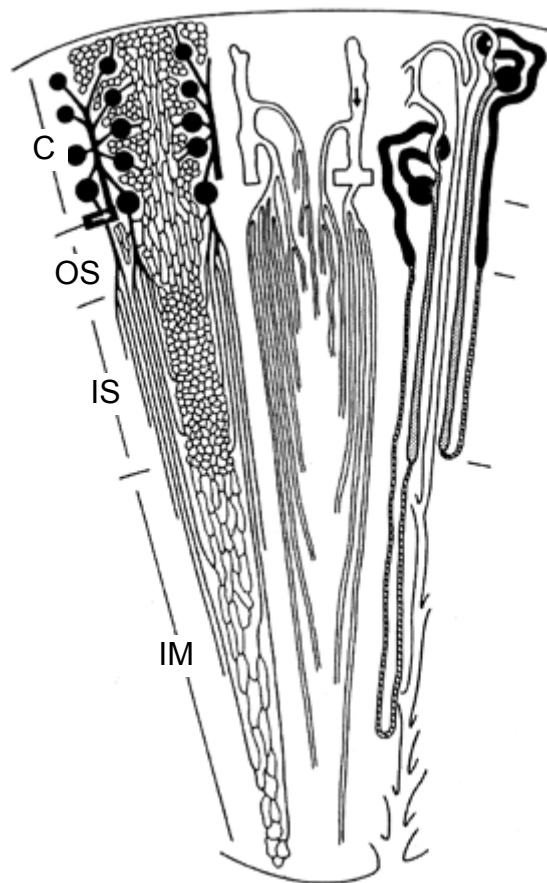


Figure 1.4: Renal microcirculation into the regions of renal cortex (C), the outer stripe of the outer medulla (OS), the inner stripe of the outer medulla, and the inner medulla (IM). Arterial vessels, descending vasa recta (DVR), and capillaries are depicted on the left; the ascending vasa recta and venous circulation are in the middle; and the renal tubular system on the right.
[adapted from Mattson, 2003]

1.1.2.a Renal regional circulation

Several functional studies have demonstrated that the blood flow in the renal medullary circulation can indeed be regulated independently of renal cortical blood flow. Such studies have shown that renal medullary blood flow increases after an elevation in renal perfusion pressure despite efficient autoregulation of renal blood flow, renal cortical blood flow, and glomerular filtration rate (Mattson *et al.*, 1993; Cowley, 1997; Evans *et al.*, 2000). The mechanisms that allow modulation of renal medullary blood flow in the absence of changes in renal cortical perfusion are not clear, but evidence obtained from both morphological and physiological studies indicates that the appropriate regulatory components exist in the medullary circulation that makes it possible for the blood flow in this part of the kidney to be selectively regulated.

The afferent and efferent arterioles are small resistance vessels that regulate renal microcirculation and glomerular filtration. Helou and Marchetti (1997) have described two subpopulations of efferent arterioles that display morphological, topological, and functional differences. Morphologically, muscular efferent arterioles (mEA) have a thick, regular, and muscular wall, and terminate as vasa rectae. The mEA of the juxtamedullary nephrons descend into the outer medulla and branch into the vasa rectae in the outer stripe of the outer medulla. These efferent arterioles contain up to four layers of smooth muscle and a layer of endothelial cells. The endothelial cell layer is continuous from the efferent arterioles to the vasa rectae, but the smooth muscle of the efferent arterioles is gradually replaced by pericytes as the vasa rectae divide and branch in the medulla (Kriz, 1981). Pericytes are cells with a phenotype similar to that observed in vascular smooth muscle (Herman and D'Amore, 1985; Nehls and Drenckhahn, 1991; Park *et al.*, 1997), and are found in both the outer and inner medulla (Park *et al.*, 1997). Cultured pericytes have been shown capable of contracting both tangentially and circumferentially (Murphy and Wagner, 1994), and this makes the pericytes ideally suited to modulate blood flow in the vasa rectae by

altering vessel diameter, and provides a mechanism by which medullary blood flow can be regulated in response to circulating hormones or locally released paracrine and autocrine factors (Mattson, 2003). On the other hand, thin efferent arterioles (tEA) have a thinner, irregular and less muscular wall, and terminate as peritubular capillaries. Based on functional aspect, in response to angiotensin II (Ang II), mEA display higher increases in intracellular Ca^{2+} concentration than tEA, but mEA are slightly less sensitive to Ang II than are tEA (Helou and Marchetti, 1997; Helou *et al.*, 2003). However, mEA from juxtamedullary nephrons in $\text{AT}_{1\text{A}}$ receptor-null mice do not exhibit a contractile response under Ang II stimulation suggesting that mouse mEA are devoid of $\text{AT}_{1\text{B}}$ receptors (Harrison-Bernard *et al.*, 2003).

Studies using isolated and cannulated DVR on vascular constrictor and dilator responses have demonstrated that the vascular diameter of the DVR can be independently altered by various vasoactive agents involving among others, changes in intracellular calcium and nitric oxide, and cell signalling pathways (Pallone, 1994; Silldorff *et al.*, 1996; Pallone *et al.*, 2000; Rhinehart and Pallone, 2001; Zhang *et al.*, 2002). These studies further establish that the regulation of blood flow within the renal medulla is independent of changes in renal cortical vascular resistance.

1.1.2.b Autoregulation of renal circulation

Renal blood flow can be altered by many conditions, among others are normal physiological changes such as exercise, pain, heat and posture, and diseases that results in decreased or loss of renal tissues, or changes in the arterioles and glomerular capillaries such as hypertension and diabetes mellitus (De Wardener, 1969). The renal microvessels have the ability to autoregulate the total and regional renal blood flow (RBF) to maintain the optimum perfusion pressure by contracting upon pressure rise and dilating upon pressure drop (Johnson and Intaglietta, 1976). This is because the intrarenal blood flow is very important for normal renal functions and any alteration to blood flow will in turn affect the renal function (Regan *et al.*, 1995).

Renal autoregulation can be defined as the kidney's ability to alter its own vascular resistance in response to changes in arterial pressure to maintain essentially constant RBF and glomerular filtration rate (GFR) over a range of arterial pressure from 90 to 180 mmHg (Cupples, 1993). Glomerular capillary pressure (P_{gc}) is a function of the balance between afferent and efferent vascular resistances, as well as the level of systemic blood pressure. Increased P_{gc} has been suggested to be a major pathogenetic factor in the development of glomerular sclerosis in hypertensive renal disease in rats (Ofstad *et al.*, 1992). Lowering of the increased P_{gc} has been shown to attenuate glomerular degeneration and preserve renal function (Anderson *et al.*, 1986; Jackson and Johnston, 1988; Brunner *et al.*, 1989; Tolins and Raij, 1990; Wenzel *et al.*, 1992; Dworkin *et al.*, 1993; Griffin *et al.*, 1994, 1995). Adjusting the renal vascular resistance, mainly involving the afferent vessels, may prevent variations in P_{gc} during everyday transitory fluctuations of the systemic blood pressure (Navar, 1978). In experimental conditions, autoregulation keeps both RBF and P_{gc} constant within a wide range of acute pressure variations. When hypertension becomes chronic, the autoregulation is reversibly reset toward higher blood pressures, whereas the capacity of autoregulation seems to be maintained (Iversen *et al.*, 1987). However, an increase in P_{gc} seems to follow long-standing hypertension (Iversen *et al.*, 1998). Among the many controllers of volume and sodium excretion (Dietz *et al.*, 2001; Gabrielsen *et al.*, 2001; Lohmeier *et al.*, 2001; Peterson *et al.*, 2001; Andersen *et al.*, 2002; Schou *et al.*, 2002), blood pressure appears to be among the most potent (Mattson, 2003). When in the well-hydrated state, the renal medullary circulation loses its capacity to autoregulate (Mattson, 2003). Accordingly, hypertension will wash out the osmotic gradient, thereby limiting the amount of fluid excreted.

Autoregulation of RBF is mediated by a rapid myogenic response and a slower tubuloglomerular feedback mechanism (TGF). The myogenic response is, to some degree, an opened-loop mechanism meaning that the local vasoaction will not directly feedback onto the input signal. Conversely, TGF operates in a closed-loop mode. The

TGF responds with a frequency of 0.02 to 0.06 Hz, and has a half-life of roughly 10 to 30 s, whereas the myogenic response is quicker, acting in a range between 0.1 and 0.3 Hz with a half-life of 1 to 4 s (Moore, 1984; Schnermann and Briggs, 1989; Holm *et al.*, 1990; Moore and Casellas, 1990; Holstein-Rathlou *et al.*, 1991; Holstein-Rathlou, 1993; Janssen *et al.*, 1995; Cupples and Loutzenhiser, 1998; Just *et al.*, 1998; Walker *et al.*, 2000; Wang *et al.*, 2000). Both mechanisms due to their different frequency properties, contribute differently to the response to slow and rapid pressure ramps. Slow pressure changes may elicit a predominant TGF response, whereas rapid pressure changes also involve the myogenic response (Flemming *et al.*, 2001).

The key feature that incorporates the TGF simply relates the distal tubule inflow to the afferent arteriolar resistance, such that increases in distal tubular inflow result in vasoconstriction while decreases in flow lead to vasodilation. The distal tubular feedback mechanism mediates autoregulatory adjustments in renal vascular resistance (Navar, 1978). The TGF is known to reset during sustained alterations of arterial pressure (Selen and Persson, 1983), and it has been demonstrated that Ang II, at concentrations that do not alter renal resistance, increases the magnitude of TGF response (Ploth *et al.*, 1979; Mitchell and Navar, 1988). Ang II blockade prevents resetting of autoregulation to operate at lower arterial pressure and RBF, and also impairs operation of the TGF (Cupples, 1993). This finding is compatible with an attenuation of TGF during ACE inhibition (Sorenson *et al.*, 2000). It has been shown that TGF responses were also absent in ACE-deficient (Traynor *et al.*, 1999) as well as in AT_{1A} receptor-deficient (Schnermann *et al.*, 1997) mice, and could be restored to near-normal levels with Ang II (Traynor *et al.*, 1999). Feldberg *et al.* (1995), using simulation studies, concluded that the myogenic response is a prerequisite to obtaining any degree of autoregulation of RBF by the TGF mechanism. On the contrary, myogenic oscillations can be present when TGF is absent (Navar, 1998).

Resetting of the RBF autoregulation serves to protect the glomerular capillaries from the variations of the systemic pressure. When arterial pressure is reduced

acutely, the magnitude of TGF response also decreases proportionally (Schnermann and Briggs, 1989) but recovers to control levels after 20 minutes despite continuing low pressure (Selen and Persson, 1983). Moreover, Holm *et al.*, (1990) have shown that release of a suprarenal aortic clamp causes a short-lived hyperaemia followed by profound renal vasoconstriction that decays over 20 minutes. The vasoconstriction could be blocked by an angiotensin converting enzyme inhibitor, and they concluded that it reflected the operation of autoregulation since the vasoconstriction was pressure induced. It has been reported that the RBF autoregulation curve was reset toward higher pressure values during acute renal sympathetic nerve stimulation in dogs (Holdaas *et al.*, 1981), hypertensive young SHR (Azar *et al.*, 1979), and the non-clipped kidney in two kidney-one clip (2K1C) hypertensive rats (Iversen *et al.*, 1986). It is possible that resetting of blood flow autoregulation is a general mode of reaction when the perfusion pressure is increased, and it is expected to be a continuous process during the development of systemic hypertension.

Renal tissue damage does influence the resetting of the autoregulation. The resetting of RBF autoregulation seems to be perfect in hypertensive young spontaneously hypertensive rats (SHR) where the glomerular capillary pressure is kept normal (Azar *et al.*, 1979, Iversen *et al.*, 1987). However, in 36-week-old SHR, the glomerular capillary pressure is slightly increased when compared with age-matched WKY (Bank *et al.*, 1983) indicating less perfect resetting of the autoregulation in SHR, possibly because of the greater tissue damage. Nephron loss increases the glomerular capillary pressure in the remnant nephrons (Azar *et al.*, 1977). The glomerular capillary pressure has also been reported to be elevated in the non-clipped kidney in the 2K1C rat (Steiner *et al.*, 1982), the DOCA-salt hypertensive rats (Dworkin *et al.*, 1982), and in the Milano hypertensive rats (Baer and Bianchi, 1978).

Animal experiments have demonstrated significantly different effects of antihypertensive drugs on RBF autoregulation. Calcium channel blockers abolish RBF autoregulation (Navar *et al.*, 1986) by eliminating the macula densa response (Mitchell

and Navar, 1990) whereas preserved autoregulation during resetting, caused by treatment with α_1 -adrenoceptor blocker and angiotensin converting enzyme inhibitor were observed (Navar *et al.*, 1986; Iversen *et al.*, 1987; Kvam *et al.*, 1998). Hence, it is important that the preferable antihypertensive agents should not only reduce arterial blood pressure but also preserve RBF autoregulation and a normal P_{gc} . Antihypertensive drugs that abolish RBF autoregulation could be deleterious to the kidney if the systemic blood pressure is not reduced sufficiently.

1.1.3 Role of the kidney in the long-term regulation of arterial pressure

The theoretical importance of the kidney in the control of arterial pressure and the concept that alterations in renal function lead to adjustments in arterial blood pressure was first introduced by Guyton and colleagues (1974). Although arterial blood pressure is controlled by many regulatory systems, it is proposed that the kidney, through its ability to regulate extracellular fluid volume, is the dominant long-term controller of arterial pressure (Guyton *et al.*, 1999). A key feature of this control system is pressure natriuresis, or the ability of the kidneys to respond to changes in arterial pressure by altering the renal excretion of salt and water (Reinhart *et al.*, 1995; Lohmeier *et al.* 2000).

The importance of the renal capacity to excrete sodium and water for the level of sustained arterial pressure of an organism has been revealed by system analyses of arterial pressure regulation (Guyton *et al.*, 1999). Support for the important role of the kidney for long-term arterial pressure regulation, and for the development and maintenance of genetic forms of arterial hypertension comes from renal transplantation studies in patients and experimental animals (Rettig *et al.*, 1993, 1996). Renal transplant studies performed between hypertensive and normotensive strains of rats have demonstrated that the long-term level of arterial pressure in the recipient is dependent on the genetic background of the donor kidney. Transplantation of the kidney from the SHR, the Dahl salt-sensitive rat, the Milan hypertensive rat, the stroke-

prone SHR, and the Prague hypertensive rat into histocompatible normotensive recipients induces arterial hypertension in the recipient (Cowley, 1992; Rettig *et al.*, 1993; Mattson and Cowley, 1999). The opposite experiments have also been successfully performed where the kidney from normotensive donor rats transplanted into bilaterally nephrectomized SHR combined with immunosuppression lowers arterial blood pressure in SHR (Patschan *et al.*, 1997).

Following transplantation of a kidney from young SHR donors into normotensive histocompatible recipients, hypertension develops within a few weeks and reaches a stable level within 6 – 8 weeks after renal transplantation (Rettig *et al.*, 1990). The development of renal post-transplantation hypertension is associated with increased renal sodium retention (Graf *et al.*, 1993). Other mechanisms involved in the pathophysiology of post-transplantation hypertension remain unclear. Studies on renal plasma flow and GFR (Rettig *et al.*, 1990), renal and plasma renin-angiotensin systems (Rettig *et al.*, 1994), and renal α -adrenoceptor density (Michel *et al.*, 1992) do not show differences in the respective traits that could be associated with this form of hypertension. Also, there is no sympathetic reinnervation of the grafted kidney during the development of renal post-transplantation hypertension, thus the effects of the recipients sympathetic nervous system are of limited importance for the development of renal post-transplantation hypertension (Grisk *et al.*, 2000).

Clinical studies have produced similar data, and it was first demonstrated that in patients who received a renal transplant from a donor with a family history of hypertension, mean arterial pressure was significantly higher than in patients whose donor family had a normotensive history (Guidi *et al.*, 1996). Whereas, patients who received a transplant from a hypertensive donor had higher blood pressures compared with patients who received kidneys from normotensive donors (Strandgaard and Hansen, 1986). Finally, transplantation of a kidney from a normotensive donor produced a sustained normalisation of arterial pressure in hypertensive patients who had demonstrated long-standing essential hypertension (Curtis *et al.*, 1983). These

clinical data emphasize the importance of the kidney in the development and maintenance of hypertension in humans and experimental animals.

Transplant studies have established the role of the kidney in arterial blood pressure regulation, but the intrinsic renal mechanisms that regulate arterial pressure are not revealed by these experiments. The kidney could influence blood pressure regulation by altering renal afferent nerve activity (DiBona and Koop, 1997), releasing vasoactive factors into the circulation (Bergstrom *et al.*, 1998), or by altering extracellular fluid volume through a number of different mechanisms (Cowley and Roman, 1996). Chronic alterations in renal adrenergic activity achieved either by renal denervation or long-term infusions of norepinephrine directly into the renal artery, alter pressure natriuresis and produce sustained changes in arterial pressure (Reinhart *et al.*, 1995; Lohmeier *et al.* 2000).

1.1.3.a Medullary circulation in the long-term regulation of arterial pressure

The renal medulla receives only a small fraction (10%) of total renal blood flow, and the flow per unit of tissue weight is only approximately 30 – 60% of that in the cortex (Pallone *et al.* 1990, 2000), yet its microcirculation appears to play a critical role in the long-term maintenance of arterial pressure via its influence on sodium and water reabsorption (Cowley, 1997; Bergstrom and Evans, 2000). This appears to be mediated mainly through the influence of medullary blood flow (MBF) on tubular reabsorption of salt and water (Cowley, 1997).

Based on pharmacological studies, it has been suggested that changes in blood flow in the renal medulla is an important mechanism in the regulation of sodium and water excretion, and dilation of the renal medullary circulation can have a natriuretic and/or diuretic effect (Lameire *et al.*, 1980; Fadem *et al.*, 1982). A direct increase in renal perfusion pressure (RPP) leads to an increased sodium and water excretion in the isolated perfused kidney (Aperia *et al.*, 1971; Tobian *et al.*, 1978), and kidneys studied *in vivo* (Roman and Cowley, 1985; Roman, 1988; Roman *et al.*, 1988;

Majid *et al.*, 1993, 1997, 1998) in the absence of measurable changes in whole kidney RBF or GFR. In addition, the hydrostatic pressure in the postglomerular capillaries in the renal cortex, the peritubular capillaries, is also constant as RPP is increased (Roman and Cowley, 1985; Roman *et al.*, 1988). However, blood flow in the vasa recta capillaries of the renal medulla was demonstrated to increase directly with RPP despite the autoregulation of GFR and RBF in normal mice, rats, and dogs (Roman *et al.*, 1988; Strick *et al.*, 1989; Farrugia *et al.*, 1993; Mattson *et al.*, 1993; Huang *et al.*, 1994; Bergstrom *et al.*, 1998; Gross *et al.*, 1998a, 1998b). The increase in flow in the vasa recta capillary bed in the renal medulla is coincident with an increase in vasa recta hydrostatic pressure (Roman *et al.*, 1988), increased renal interstitial hydrostatic fluid pressure (Roman *et al.*, 1988; Garcia-Estan and Roman, 1989; Patel *et al.*, 1994), decreased reabsorption of sodium and water from the proximal segments of deep nephrons (Haas *et al.*, 1986; Roman, 1988), and increased excretion of sodium and water (Roman and Cowley, 1985; Roman *et al.*, 1988).

Experiments by Mattson *et al.*, (1994) and Makino *et al.*, (2002) have shown that a selective and sustained decrease in medullary perfusion can lead to retention of sodium and development of hypertension. Continuous renal medullary interstitial infusion of L-NAME in the normotensive Sprague-Dawley rats significantly decreased renal medullary blood flow by 30%, and was accompanied by a significant retention of sodium, an increase in body weight, and the development of hypertension. Renal medullary blood flow returned to normal levels, a negative sodium balance was observed, and blood pressure returned to levels not different from control when the interstitial L-NAME infusion was discontinued (Mattson *et al.*, 1994) without any change in renal cortical blood flow (Makino *et al.*, 2002).

Changes in resistance in renal cortical vessels can also have a profound impact on blood flow to the medulla as any change in inflow resistance will be reflected in blood flow in the downstream segments of the renal medulla, and the likely sites involved are the preglomerular resistance vessels, i.e. the afferent arterioles (Mattson,

2003). In summary, the medullary circulation is important in the long-term regulation of fluid and electrolyte balance, and blood pressure. Direct long-term alterations in renal medullary blood flow can lead to changes in sodium and water excretion which may be translated into sustained alterations in sodium balance and a new level of arterial blood pressure.

1.1.3.b Influences of Ang II on renal medullary circulation.

Renal vessels, in general, and especially in SHR are more sensitive to catecholamines, Ang II, and also calcium channel blockers (CCBs) than the systemic resistance vessels (Loutzenhiser and Epstein, 1990). The CCBs affect mainly the afferent arteriole (Fleming *et al.*, 1987; Cacellas and Moore, 1990) and so do the α_1 -adrenoceptor blocker. Both, Ang II and catecholamines, have been shown to constrict preglomerular vessels in isolated arteries (Edwards, 1983), in isolated kidneys (Steinhausen *et al.*, 1990), and *in vivo* (Chatziantoniou and Arendshorst, 1992). Similar to Ang II, nervous stimulation has been reported to affect both afferent and efferent resistances (Hermansson *et al.*, 1981). The effect of nervous stimulation also be facilitated by increased Ang II levels as Ang II is an important mediator of the contraction of resistance vessels during nervous stimulation in rats (Pelayo *et al.*, 1984).

Ang II is a major mediator of the regulation of glomerular filtration through its combined control of the vascular tone in both arterioles. Ang II has been shown to have a vasoconstrictor effect on the renal medullary circulation (Faubert *et al.*, 1987; Pallone, 1994), no influence on the medullary circulation (Mattson *et al.*, 1991; Mattson and Roman, 1991), or even an increase in medullary blood flow with high doses of Ang II (Nobes *et al.*, 1991). Prostaglandin (Cupples *et al.*, 1988; Mattson and Roman, 1991; Pallone, 1994), nitric oxide (Zou *et al.*, 1997; Szentizvanyi *et al.*, 1999, 2002), and kinins (Nobes *et al.*, 1991) attenuate the vasoconstrictor action of Ang II in the medullary circulation. Szentizvanyi *et al.* (2002) reported an inherited defect in the

ability of Dahl salt-sensitive rat to produce nitric oxide within the outer medulla of the kidney along with a failure of medullary nitric oxide concentrations to increase in response to Ang II in this strain. As a result, small elevations of circulating Ang II that have no effect in normal rats lead to hypertension in Dahl salt-sensitive rats.

The net effect of Ang II on blood flow in the renal medulla *in vivo* appears to be due to increased resistance in the efferent arterioles of the juxtamedullary nephrons (Carmines *et al.*, 1986) and/or due to direct effects of Ang II to constrict the DVR (Pallone, 1994). Yet, the net effect of changes in circulating Ang II in the physiological range on medullary blood flow in conscious rats is minimal (Gross *et al.*, 1998), possibly due to antagonistic actions of different vasodilatory factors.

In addition, the mechanism of the decrease in medullary blood flow appears to be due to a combination of a reduction in renal cortical blood flow (Leonard *et al.*, 2000) as well as direct actions of norepinephrine to vasoconstrict the DVR (Yang *et al.*, 1995). Pharmacological studies have demonstrated that the sympathetic neurotransmitter norepinephrine decreases renal medullary perfusion via stimulation of α_1 -adrenoceptors (Zou and Cowley, 2000).

1.1.4 Renal innervation

The sympathetic nervous system (SNS) plays an important role in arterial pressure regulation. This system regulates the total and regional peripheral resistance and capacitance of the vascular system mainly through changes following release of catecholamines from both the sympathetic nerve terminals and the adrenal medulla gland (Guimaraes and Moura, 2001), and binding of the catecholamines to the adrenergic receptors.

The mechanisms by which the SNS regulates arterial pressure acutely and chronically are quite different (Guyton, 1980), hence, acute blood pressure responses to either sympathetic stimulation or inhibition may not be generalised into the contribution of the SNS to the chronic maintenance of arterial pressure. The activity of

the SNS is important for rapid adjustments of cardiovascular and renal function in response to changing environment conditions. Its involvement in long term arterial pressure regulation remains unclear due to a lack of reliable methods or technical limitations to chronically quantify sympathetic activity, and adaptation of the nervous system to repeatedly applied stimuli, for example, central and peripheral resetting of baroreflexes, and to determine the sustained influence of the sympathetic nervous system on sodium excretion (Lohmeier *et al.*, 2001). Numerous acute studies demonstrating that Ang II actually increases sympathetic activity (Reid, 1992; Fink, 1997), but yet it is fascinating to discover that chronic baroreflex-mediated suppression of renal sympathetic nervous activity (RSNA) is the compensatory response to Ang II hypertension leading to attenuation of the antinatriuretic and hypertensive effect of Ang II (Carroll *et al.*, 1984; Cox and Bishop, 1991; Lohmeier *et al.*, 2001). However, there is little chronic data to support the contention that the sympathetic nervous system contributes to Ang II hypertension (Reid, 1992; Fink, 1997).

In addition to short lasting effects on organ function, the sympathetic nervous system may also induce chronic effects on target organs, manifested as altered DNA, protein synthesis and changes in organ morphology. SHR are characterised by increased sympathetic innervation of their internal organs compared to normotensive animals. Selective surgical renal denervation in young SHR delays, but does not prevent, the development of arterial hypertension, and complete neonatal sympathectomy induces a long term reduction of arterial pressure in SHR. Hence, of how reduction in sympathetic tone affects long term blood pressure in SHR is dependent on the stage during ontogeny at which it is applied, and on the extent to which sympathetic influences are removed. The specific organ affected is also a contributing factor. Since the kidney appears to be the major determinant of long term blood pressure in SHR, studying the specific role of early sympathetic activation within this organ for the development of hypertension in this model is a prospect to consider.

The kidney has very dense sympathetic innervations, and neuroeffector junctions have been described along most of the vascular and tubular elements of the tissue (Coote *et al.*, 1972; Johns *et al.*, 1976). Renal sympathetic nerve activity (RSNA) plays a significant role in the regulation of renal haemodynamics and excretory function (Malpas *et al.*, 1996; DiBona and Koop, 1997; Malpas and Evans, 1998; DiBona, 2000; Leonard *et al.*, 2001). Renal nerves modulate RBF variability in several physiological conditions, such as exerting an antihypertensive effect when renal perfusion pressure is reduced, and RSNA is increased during hypoxia or hemorrhage (Janssen *et al.*, 1997; Malpas *et al.*, 1998, 1999; Nafz *et al.*, 2000; Zou and Cowley, 2000; Barret *et al.*, 2001).

At a functional level, high levels of direct electrical stimulation of the renal sympathetic nerves resulted in frequency related decreases in RBF (Sattar and Johns, 1994a, 1994b; Armenia *et al.*, 2004), GFR and renin release (Coote *et al.*, 1972 and Johns *et al.*, 1976). One of the groundbreaking reports was that of LaGrange and his co-workers (1973) who observed that electrical stimulation of the renal nerves at low levels, which had no measurable impact on renal haemodynamics, still caused a large decrease in sodium and water excretion. These observations laid the foundation for the concept that the renal nerves could directly act on the tubular epithelial cells of the proximal tubule (Bello-Reuss *et al.*, 1976) and thick limb of the loop of Henle (DiBona and Sawin, 1982) to increase the rate of fluid transport from lumen to interstitium. Currently, it is considered that graded activation of the renal sympathetic nerves progressively recruits a number of functions, initially stimulating renin release from the granular juxtaglomerular cells; thereafter sodium absorption is raised; while at the highest rates reductions in blood flow and glomerular filtration occur (Osborn and Johns, 1989; DiBona and Kopp, 1997). Together, these reports support the view that the neural control of sodium reabsorption by the kidney is exerted at levels that have minimal effects on renal haemodynamics.

RSNA has been shown to be important in the control of total RBF (Malpas *et al.*, 1996; Malpas and Evans, 1998), yet its role in the control of regional kidney blood flow remains unclear. RSNA may have a profound effect on the long-term control of arterial pressure mediated via effects on the medullary microcirculation. In addition to the multiple effects of sympathetic nerve stimulation on kidney function (DiBona and Koop, 1997), renal nerve stimulation differentially decreases blood flow in the renal cortex and medulla (Rudenstam *et al.*, 1995; Leonard *et al.*, 2000, 2001). The renal nerves might differently regulate cortical (or total renal) blood flow (CBF) and MBF through different frequency response characteristics of the vasculature in these two regions. MBF appears to be less sensitive to sympathetic nerve activity than CBF, regardless of whether the nerves are activated by electrical stimulation (Leonard *et al.*, 2000) or reflexively (Leonard *et al.*, 2001), but sensitivity within these vascular territories appears to be relatively homogenous (Guild *et al.*, 2002).

The medullary insensitivity is particularly evident at relatively low stimulus intensities, as might occur under physiological conditions (Rudenstam *et al.*, 1995; Leonard *et al.*, 2000, 2001) despite juxtamedullary efferent arterioles (mEA) having more layers of smooth muscle cells (Kriz, 1981; Helou and Marchetti, 1997; Pallone *et al.*, 1998), and denser innervation (Gorgas, 1978) than superficial or outer cortical efferent arterioles. This may be due to the influence of α_2 -receptor stimulation by norepinephrine in the medulla to increase the release of NO, which opposes the vasoconstrictor effects of norepinephrine (Zou and Cowley, 2000). Also, differences in the density and organization of vascular network and vascular structure (Kriz, 1981; Pallone *et al.*, 1990), density and distribution of innervations (Barajas and Powers, 1990), or the kinetics of smooth muscle contraction between cortical and medullary vascular sites may result in a difference in the ability to respond to the different frequencies in RSNA.

The renal sympathetic nerves represent one primary mechanism by which kidney fluid reabsorption can be modulated, and via the consequent increase or

decrease in extracellular fluid volume can have a major impact on blood pressure. The renal nerves appear to be the critical link between the SNS and long-term regulation of arterial pressure, because they impact renal excretory function (Guyton, 1980). Sympathetic nervous system responds chronically, as well as acutely, to regulate body fluid volumes and arterial pressure. It has been demonstrated that suppression of RSNA and attendant increments in renal excretory function are long-term responses to excess body fluid volumes and hypertension (Lohmeier and Hildebrandt, 1998; Lohmeier *et al.*, 1998, 1999, 2000). During high salt intake or hypertension induced by chronic intravenous infusion of either norepinephrine or Ang II, there is a relative increase in sodium excretion from innervated as compared to denervated kidneys (Lohmeier and Hildebrandt, 1998; Lohmeier *et al.*, 1998, 1999, 2000). This indicates that RSNA is suppressed in these states of chronic volume excess and/or hypertension.

It has also been determined that the chronic suppression of RSNA in Ang II hypertension is abolished by denervation of sinoaortic and cardiopulmonary baroreceptors, suggesting that baroreflexes play a critical role in the long-term response (Lohmeier *et al.*, 2000). Subjecting rats to coarctation of the aorta to produce an abrupt and constant increase in arterial pressure, and from recording of aortic baroreceptor activity during anaesthesia, Krieger (1986) concluded that complete baroreceptor resetting occurs within 48 hr of hypertension. Another study by Cowley and DeClue (1976), by inducing a rapid and sustained increase in arterial pressure by chronic infusion of Ang II in dogs with and without sinoaortic denervation, showed that acute increases in arterial pressure in response to Ang II were more pronounced after deafferentation of arterial baroreceptors than before when the arterial baroreflex was intact. However, the increase in blood pressure was equal in both groups, and finally the arterial pressures on day 7 of Ang II infusion were comparable. Thus, the baroreceptors appeared to have no sustained influence on the severity of the